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# **Content Determination of Functional Composition and Antioxidant Activity from Six Purple Plants**

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#### ABSTRACT

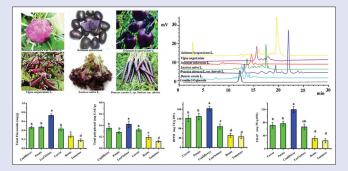
Background: Nowadays, many purple vegetables are available in the market. These vegetables have rich content of anthocyanins, which gives them their characteristic purple color. A high intake of anthocyanin-rich vegetables/plant parts imparts potential beneficial effects. Therefore, in this study, we investigated six purple plants (SPPs; purple cauliflower, purple potato, purple lettuce, purple carrot, purple beans, and purple tomato) for their content of functional composition and their antioxidant activity. Materials and Methods: Anthocyanins from SPPs were extracted and purified by an optimized process. Then, we determined the total anthocyanin (TA) and cyanidin-3-O-glucoside (C-3-G) content in the anthocyanin purified extracts (APEs) from the SPPs through pH difference and high-performance liquid chromatographic methods, respectively. 2,2-diphenyl-1-picrylhydrazyl and ferric reducing antioxidant power methods were used to test the antioxidant activities of the APEs. In addition, the contents of the total flavonoids, total polyphenol, Vitamin C, and dietary fiber from the SPPs were determined. Results: SPPs are rich in anthocyanins, flavonoids, phenolic acids, and other compounds that show strong antioxidant activity. According to our results, anthocyanin content was the highest in purple cauliflower, which translated into the greatest antioxidant activity of the APEs of purple cauliflower. The content of total flavonoid, polyphenol, and Vitamin C in the purple lettuce was higher than that of the other plants. The highest content of dietary fiber was found in purple potato. Conclusion: According to our results, purple cauliflower is the most potential plants to the pharmaceutical industry. Our results provide a theoretical basis for further exploration of efficacy of the purple plants and exploitation.

Key words: Anthocyanin, antioxidant activity, functional composition, high performance liquid chromatography, purple cauliflower

#### **SUMMARY**

• Contents of total anthocyanin and functional components and antioxidant activity from *Brassica oleracea* var. *botrytis* L. are the highest in six purple vegetables

• Brassica oleracea var. botrytis L. with high antioxidant activity is also a promising source of anthocyanins.



**Abbreviations used:** APEs: Anthocyanin purified extracts; SPPs: Six purple plants; HPLC: High performance liquid chromatography; TA: Total anthocyanin; C-3-G: Cyanidin-3-O-glucoside; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: Ferric reducing antioxidant power; DW: Dry weight; FW: Fresh weight; TEAC: Trolox equivalent antioxidant capacity; Vc: Vitamin C.

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# **INTRODUCTION**

There is a rising concern over the increase in the incidence of modern lifestyle diseases such as hypertension, hyperlipidemia, diabetes, and coronary heart disease. Therefore, plants rich in functional components are receiving more and more attention by various research groups, especially the purple plants. Purple plants are rich in functional compounds such as anthocyanins, flavonoids, polyphenols, dietary fiber, and Vitamin C, which show antioxidant and anti-inflammatory activity and improve metabolism, reduce diabetes, and provide positive health benefits to human health.<sup>[1]</sup> Anthocyanins, a class of flavonoids, are widely present in the petals, fruits, stems, and leaves of plants. Its color changes with the change in pH; therefore, the intensity of the color in various plant parts is positively correlated with the content of anthocyanins.<sup>[2]</sup> Anthocyanins show strong antioxidant activity due to its special structure [Figure 1] and studies have shown that it is one of the strongest antioxidants ever found.<sup>[3]</sup> In addition, studies have confirmed

that anthocyanins show strong, anti-aging, antibacterial, antidiabetic, anti-asthenopia, and neuroprotective effects.<sup>[4]</sup> Nowadays, obtaining purple plants has become a new pursuit for various agriculturists. In order to meet the demand, researchers have cultivated variety purple or black plants, such as black corn, purple grape, and purple pepper. With the emergence of more and more purple plants, it is important

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R	Anthocyanidin	R <sub>1</sub>	R <sub>2</sub>
З' .он	pelargonidin	н	н
2' 1 4'	cyanidin	OH	н
0 7 8 10 <sup>+</sup> 2 1 B	peonidin	OCH <sub>3</sub>	н
$\left[ \begin{array}{c} A \end{array} \right] \left[ \begin{array}{c} c \end{array} \right]_{3} = \begin{array}{c} \delta & \delta' \cdot \mathbf{R}_{2} \\ \delta & \delta' \cdot \mathbf{R}_{2} \end{array}$	delphinidin	он	OH
6 5 4 OH	petunidin	OH	OCH <sub>3</sub>
он	malvidin	OCH <sub>3</sub>	OCH <sub>3</sub>

Figure 1: The structure of 6 common anthocyanins

to study their efficacy and nutritional qualities. In this study, the emergence-cultivated six purple plants (SPPs) were selected to study the content of anthocyanin. In addition, we assessed the antioxidant activity of the anthocyanin purified extracts (APEs) and the total content of flavonoids and other functional components to evaluate their health efficacy and nutritional value.

### **MATERIALS AND METHODS**

#### Instruments and chemicals

In this study, we used Waters 2695 High-Performance Liquid Chromatograph (Waters 2996 PDA Diode Array Detector, Empower Chromatographic Work Station) and Waters XBridge C. Chromatographic Column (Waters Corp, USA). Infinite M2000 Microplate Reader (Swiss Tecan), WFZ UV-2000 Ultraviolet and Visible spectrophotometer (Shanghai Unico Instrument Corp., Ltd.), PHS-3C pH meter (Shanghai Precision Scientific Instrument Co., Ltd.), KQ-250B Ultrasonic cleaner (Kunshan Supersonic Instrument Corp., Ltd.), R201B rotary evaporimeter (Shanghai Yarong Biotechnological Corp., Ltd.), JHBE-50S smashing tissue triturator (Henan Jinding Development Co. Ltd.) were also used. Standard sample of cyanidin-3-O-glucoside (C-3-G) was purchased from Biopurify Phytochemicals Ltd.(Sichuan, China); Trolox; 1,3,5-tri (2-pyridyl)-2,4,6-triazine (TPTZ); and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Aladdin Biotechnology Co., Ltd. Diaion HP 2 MGL microporous resin were purchased from Mitsubishi in Japan. All chemical reagents were of analytical grade. Rutin, gallic acid, ascorbic acid,  $\alpha$ -amylase, protease, and amyloglucosidase were purchased from Sinopharma Chemical Reagent Co. Ltd.

#### **Plant materials**

Purple beans (*Vigna unguiculata* L.), purple tomato (*Solanum lycopersicum* L.), purple leaf lettuce (*Lactuca sativa* L.), purple potato (*Solanum tuberosum*), purple carrot (*Daucus carota* L. sp. *Sativus* var. *atrorubens.*), and purple cauliflower (*Brassica oleracea* var. *botrytis* L.) were randomly harvested from planting bases [Figure 2]. The harvest time of the plants depends on the maturity period of the analyzed species. Approximately, 1000 g of each sample was immediately placed in an airtight preserving box containing ice bags.

# Preparation of the anthocyanin purified extracts from the six purple plants

Anthocyanins were extracted and purified by an optimized process. Briefly, 500 g of SPPs were accurately weighed and placed in a crushing extractor (fabricated in our laboratory). To this, we added 60% ethanol with a material: Liquid ratio of 1:5 and the pH was maintained at 2. The crushing extraction was continued for 3 min. Then, the mixture was filtered and the residue was weighed again. Next, the ratio of material-liquid was maintained at 1:6 and the extraction was conducted in an ultrasonic extractor for 40 min. Then, the material was collected and concentrated at 40°C by the process of flash-distillation until there was no alcoholic taste. The concentrated solution was adsorbed and enriched by the macroporous resin column chromatography of Diaion HP 2 MGL. After 30 min of saturated adsorption, elution was performed with deionized water equal to 5 times the volume of the column until the effluent is clarified and then elution the red band with 60% ethanol at pH 3 until the effluent was colorless. The purple-red eluate was collected and concentrated at 40°C under reduced pressure to obtain the dry powder of APEs, which was stored at low temperatures and in a dry place for future use.

# Content determination of the total anthocyanin and the cyanidin-3-O-glucoside

The content of TA in APEs was determined by pH differential method.<sup>[5]</sup> Briefly, APEs (20 mg) was dissolved in 10 mL of 10% alcohol. Then, 1.0 mL of sample solution (1.0 mg/mL) was mixed with 9 mL of the buffer (pH 1 and pH 4.5, respectively). The absorbance values at 510 nm and 700 nm respectively were measured after 1 h in the dark, and the content of total anthocyanin (TA) was calculated by the following equation:

TA (w/w) =  $(A \times M \times DF \times V)/(\epsilon \times L \times Wt)$ 

Where TA is the TA, A = (A510 nm – A700 nm at pH 1.0) – (A510 nm – A700 nm at pH 4.5); M is the molecular weight, DF is the dilution factor, V is the volume,  $\varepsilon$  is the extinction coefficient, L is the optical path, and Wt is the plant weight.

The content of the C-3-G was determined by High performance liquid chromatography (HPLC).

#### Preparation of sample solution

APEs from each plant were weighed accurately and dissolved in 10 mL of 2% HCl methanol solution.

#### Preparation of reference solution

Briefly, 2.0 mg of the C-3-G reference substance was accurately weighed and dissolved in 10 mL of 2% HCl methanol solution.

#### High performance liquid chromatography condition

In this study, we used Waters XBridge  $C_{18}$  Chromatographic Column (4.6 mm × 250 mm, 5 µm). The column temperature was maintained at 30°C, with a flow rate of 1.0 mL/min and a detection wavelength of 520 nm. Acetonitrile (A) and 0.1% phosphoric acid water (B) was used as the mobile phases. The gradient was maintained as follows: 0–20 min, 5%–20% A and 20–30 min, 20% A. The samples were dissolved in 2% methanol, and all samples were filtered through a 0.45 µm membrane before injection. The injection volume was 10 µL. The standard curve was plotted based on the values obtained for 5, 10, 15, 20, and 25 µL of the reference solution to measure the peak area according to HPLC chromatography conditions. The standard curve was drawn with the content of the C-3-G as the abscissa (X) and the peak area value as the ordinate (Y), and the regression equation was Y = 55554.9 X – 3141.34 ( $R^2 = 0.9998$ ).

# Determination of the total flavonoid and total phenolic content

To determine the content of total flavonoids, 10.0 g of the plant material was accurately weighed, cleaned, and ground using a mortar and pestle. To this material, 70% ethanol was added at a ratio of 1:20 (W:V). Then, the phenolics were extracted in an ultrasonic water bath maintained at 40°C for 50 min. The contents were centrifuged, and the supernatant was collected for analysis. Briefly, 1 mL of the sample solution was added to



Figure 2: The selected six purple plants in the experiment

1 mL of 5% NaNO<sub>2</sub> solution. After 6 min, 1 mL of 10% Al (NO<sub>3</sub>)<sub>3</sub> was added to the reaction mixture. Subsequently, 3 mL of 1 mol/L NaOH solution was added to the reaction mixture and the volume was adjusted to 10 mL with 70% ethanol. The absorbance was measured at 510 nm along with the reference standard, and the results are expressed as rutin equivalents (mg RE/g). The linear range of the standard sample was 0.1–0.6 mg/mL,  $R^2 = 0.9993$ .

#### Content determination of the total polyphenol

Briefly, 5.0 g of the plant material was accurately weighed, cleaned, and ground using mortar pestle. Then, we added 65% ethanol at a ratio of 1:15 (W:V) and the contents were extracted using ultrasonic extractor at 50°C for 30 min. This extract was centrifuged, and the supernatant was collected for further experiments. To 1 mL of the sample solution, we added 0.3 mL of Folin–Ciocalteu reagent and 2 mL of 10% Na<sub>2</sub>CO<sub>3</sub>. The volume was adjusted to 10 mL with distilled water and the reaction was allowed to continue in the dark. After 1 h, the absorbance was measured at 760 nm using a UV-Vis spectrophotometer. Gallic acid was used as a reference standard, and the results are expressed as gallic acid equivalents per gram of sample (mg GAE/g). The linear range of the standard sample was 10–100 µg/mL, and the measured  $R^2$  was 0.9993.

#### Estimation of dietary fiber and vitamin C content

#### Estimation of dietary fiber

The content of dietary fiber was estimated by following Huang Meiying's method.<sup>[6]</sup> Briefly, the sample was dried and pulverized and passed through a 100-mesh sieve. Briefly, 5 g sample was weighed accurately and added to 200 mL of 0.4 mol/L trimethylol aminomethane and ethanesulfonic acid solution. The mixture was stirred on a magnetic stirrer until the sample was completely dispersed in the buffer solution. Then, 50  $\mu$ L of  $\alpha$ -amylase was added to the sample solution and stirred slowly for the reaction to progress. After 35 min at the 95°C, the gelatinous substance was removed and washed with 10 mL of water. To this, 100  $\mu$ L of protease solution was added. After 30 min, 3 mol/L acetic acid solution was added, and the pH was adjusted pH to 4.5 ± 0.1. Then, we added 100  $\mu$ L of amyloglucosidase and the reaction was continued for 30 min. To this, 95% ethanol was added to each enzymatic

hydrolysate, and the volume ratio of ethanol to sample liquid was 4:1 and the sediment was taken out after 1 h. The sediment was spread out on ethanol-wetted diatomaceous earth. The ethanol was removed by filtration, and the sediment were transferred to a crucible and filtered and washing. Then, the residue was washed with ethanol and acetone respectively, and the residue was dried at 105°C in an oven and weighed.

#### Determination of the Vitamin C content

Briefly, 5 g of edible portion of fresh plants was weighed and added to the juice extractor. To this, we added 100 mL of 2% oxalic acid and was quickly ground into a homogenate. Then, we collected the supernatant after centrifugation for further analysis. Vitamin C was measured by the 2,6-dichloroindophenol titrimetric method.<sup>[7]</sup> Briefly, 104 mg of sodium bicarbonate was dissolved in 400 mL of distilled water. Then, 50 mg of 2,6-dichloroindophenol was weighed and placed in a 250 mL volumetric flask. After dissolving it, the solution was filtered, and stored in a brown bottle under refrigerated conditions. The solution was calibrated with vitamin C standard solution before each use.

### Determination of the antioxidant activity

#### 2,2-diphenyl-1-picrylhydrazyl assay

DPPH assay was performed based on the method of Bolling *et al.*<sup>[8]</sup> with some minor modifications. We used Trolox as the reference standard. Briefly, 50  $\mu$ L of 0.36 mg/L DPPH solution was added to 200  $\mu$ L of the sample or Trolox solution and mixed for 30 s. The reaction was allowed to continue in dark for 45 min at room temperature. Then, the absorbance was read on a 96-well microplate reader at 517 nm. The antioxidant capacity was expressed as trolox equivalent antioxidant capacity (TEAC).

#### Ferric reducing antioxidant power assay

The ferric reducing antioxidant power (FRAP) analysis was performed based on the method of Popovic *et al.*<sup>[9]</sup> FRAP solution was prepared by mixing 0.1 mol/L acetate buffer, 10 mmol/L TPTZ, and 20 mmol/L ferric chloride solution at a ratio of 10:1:1: (volume ratio). Briefly, 300  $\mu$ L of FRAP solution and 10  $\mu$ L of sample solution were mixed for 10 min and the absorbance was measured at 593 nm. With Trolox as a

positive control, the TEAC of the sample was calculated to express the antioxidant capacity.

### Statistical analysis

All data are expressed as mean  $\pm$  standard deviation. All data were analyzed using SPSS software (version 19.0, Chicago, IL, USA). We performed *t*-test in the one-way analysis to analyze the differences between groups. *P* < 0.05 was considered significantly different, and *P* < 0.01 was considered the extremely significant different. The bar graphs were drawn using Origin 17.0 (Chicago, IL, USA).

# **RESULTS AND DISCUSSION**

# The content of the TA and cyanidin-3-O-glucoside in anthocyanin purified extracts

Table 1 shows the contents of the TA in the SPPs of fresh weight (FW) and in the APEs. The content of TA in the SPPs was between  $0.042 \pm 0.011$ and  $1.037 \pm 0.042$  mg/g FW. Among the SPPs, the content of the TA was the highest in purple cauliflower. The content of TA of the APEs from the SPPs of dry weight (DW) was between  $22.53 \pm 4.25$  and  $277.12 \pm 15.31$  mg/g DW. Among the SPPs, the content of TA was the highest in the APEs from purple cauliflower and lowest in the purple tomato. The American Dietetic Association has pointed out that the daily intake of anthocyanin for humans is about 180-225 mg/day<sup>[10]</sup> and the daily intake of plants recommended by China's "National Dietary Guidelines" is about 300-500 g/day; therefore, we know that except for purple beans and purple tomatoes, the other plants can satisfy the daily intake of anthocyanin. The common variety of the SPPs is basically free of anthocyanins and when the breed was modified the content of anthocyanins greatly increased.

Dry weight is the weight of APEs; FW is the weight of purple plants extract.

Figure 3 and Table 2, respectively, show the content of the C-3-G in the APEs and the HPLC chromatogram, respectively. According to the results, the content of C-3-G in the APEs was less. The highest among them was purple potato, whose content was 32.37 mg/g DW which accounted for 20.01% of the TA. It was reported that the major components of anthocyanin in purple cauliflower were cyanidin-3-(6-sinapyl)-sophoroside-5-glucoside,

Table 1: Content of the total anthocyanin from the six purple plants

cyanidin-3-sophoroside-5-glucoside, and cyanidin-3-(6-*p*-coumar yl)-sophoroside-5-glucoside,<sup>[11]</sup> and the main ingredients in purple potatoes were pelargonium-3-ferulyl-rutino-5-glucoside, pelargonium-3-coumaroyl-rutino-5-glucoside, pelargonium-3-rutinoside, and pae oniflorin-3-caffeoyl-rutinosyl-5-glucoside.<sup>[12]</sup> However, the primary ingredients of anthocyanin in different varieties of purple plants were also different. The major components that were identified in purple carrot were cyanidin-3-galactosyl-glucoside-ferulic acid, cyanidin-3-galactosyl-xylosyl-glucosyl-erucic acid.<sup>[13]</sup>

# The content of the total phenolic acid and total flavonoid

Polyphenols are one of the secondary metabolites of plants. They are widespread in the plant kingdom and have different properties and structures.<sup>[14]</sup> Phenol hydroxyl radicals are combined with macromolecules such as proteins to play physiological functions such as the antioxidants. Polyphenols have a defensive effect. They play a particularly important role in protecting plants from the invasion of micro-organisms.<sup>[15]</sup> Literature shows that polyphenols have various biological activities such as hypoglycemic, hypolipidemic, and antidiabetic.<sup>[16]</sup>

Flavonoids are polyphenolic constituents of plants, which have a variety of biological activities and have always been the focus of

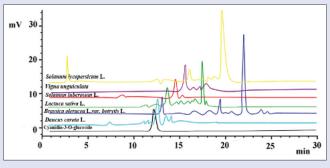


Figure 3: The high performance liquid chromatography chromatography of the anthocyanin purified extracts from the six purple plants

Plant materials	Content of t	Content of the TA (mg/g)	
	DW	FW	
Brassica oleracea var. botrytis L.	277.12±15.31ª	1.037±0.042ª	0.61
Solanum tuberosum	161.73±12.55 <sup>b</sup>	$0.815 \pm 0.026^{b}$	0.64
Daucus carota L. sp. Sativus var. atrorubens	159.35±13.58 <sup>b</sup>	0.787±0.033b	0.89
Lactuca sativa L.	92.11±10.15°	0.481±0.020°	0.55
Vigna unguiculata L.	$28.02 \pm 5.03^{d}$	$0.125 \pm 0.027^{d}$	0.45
Solanum lycopersicum L.	$22.53 \pm 4.25^{d}$	$0.042 \pm 0.011^{d}$	0.13

DW: Weight of APEs; FW: Weight of purple plants extract. TA: Total anthocyanin; DW: Dry weight; FW: Fresh weight; APEs: Anthocyanin purified extracts

Plant materials	Content of the C-3-G (mg/g DW)	As a percentage of the TA (%)
Brassica oleracea var. botrytis L.	6.37	2.30
Solanum tuberosum	32.37	20.01
Lactuca sativa L.	2.80	3.03
Daucus carota L. sp. Sativus var. atroruben	20.31	12.74
Vigna unguiculata L.	3.24	11.56
Solanum lycopersicum L.	1.29	5.75

TA: Total anthocyanin; DW: Dry weight; C-3-G: Cyanidin-3-O-glucoside

research by pharmacologists.<sup>[17]</sup> Studies have shown that flavonoids have antioxidant, anti-inflammatory, antiviral, and immune regulation activity.<sup>[18]</sup> It has found application in the field of drug development and nutraceuticals. Figure 4 shows that the content of the total flavonoids and total polyphenols in the purple lettuce was the highest, which were 0.73  $\pm$  0.034 mg RE/g and 0.42  $\pm$  0.055 mg GAE/g, respectively. These compounds were relatively low in the purple tomato, which was respectively 0.18  $\pm$  0.031 mg RE/g and 0.12  $\pm$  0.010 mg GAE/g. The experimental results showed that SPPs were not only rich in anthocyanins but also contained a large amount of the total flavonoids and total polyphenols.

### The content of the Vitamin C and dietary fiber

Vitamin C is a water-soluble vitamin whose deficiency can lead to scurvy.<sup>[19]</sup> It is an essential nutrient in human diet, and it plays an important role in regulating growth, reproduction, and the immune response of stressors. Vitamin C is abundant in brain tissue and can be involved in nerve regulation. It is a reducing agent, it can relieve the pathological symptoms of central nervous system diseases such as epilepsy, cerebral ischemia, and cerebral edema.<sup>[20]</sup> As it cannot be synthesized by the body, it must be consumed from the outside and a vegetable diet is the main way to supplement the vitamin.<sup>[21]</sup>

Dietary fiber is indigestible due to the presence of nonstarch polysaccharides, fructans, resistant starch, and lignin.<sup>[22]</sup> It is not absorbed by the body and does not generate heat, but it has high satiety value due to which it is the best choice for people with diabetes.<sup>[23]</sup> It is called the seventh essential nutrient for the human and has a potential prebiotic effect on human health. It is well known that the concept of prebiotics is rapidly expanding in contemporary medicine. Prebiotics induce the occurrence of immune responses by regulating the activity of gastrointestinal micro-organisms and leading to the formation of short-chain fatty acids, which produce a variety of physiological effects in the host such as regulating intestinal bacteria, stimulates gastrointestinal peristalsis, and so on. Therefore, it significantly alleviates colitis and constipation.<sup>[24]</sup> The content of the vitamin C in the SPPs was found to be between  $7.45 \pm 1.25$  and  $155.42 \pm 11.21$  mg/100 g FW, which was the highest in the purple lettuce. The content of dietary fiber was between  $0.81 \pm 0.12$  g/100 g and 29.86  $\pm 0.21$  g/100 g, which was found to be the highest in purple potato [Figure 5].

# Antioxidant activity of the anthocyanin purified extracts

Free radicals are oxygen molecules with unpaired electrons, which have high chemical activity and react in the form of a chain reaction.<sup>[25]</sup> Within the body, excessive free radicals can react with other substances thereby damaging various tissues and organs.<sup>[26]</sup> Modern medical research proves that many diseases such as degenerative diseases, cardiovascular diseases, cerebrovascular diseases, and inflammation are related to the excessive amounts of circulating free radicals.<sup>[27]</sup> Therefore, it is very important to treat these diseases by attempting to scavenge excess free radicals from the body. Anthocyanin is an electron donor, and it can be paired with electrons in free radicals to achieve the purpose of scavenging free radicals.<sup>[4]</sup> Anthocyanins have strong antioxidant and free radical scavenging ability because they belong to the flavonoid group. Extreme environmental pollution, pesticides, and unhealthy eating habits lead to the excessive production of free radicals, but if they are not cleared from the system, then they might damage other tissues and cells, which in turn leads to disease formation. Therefore, supplement our diet with anthocyanins might help to clear out free radicals. Purple plants show different levels of antioxidant activities. In this study, the antioxidant activity was measured using DPPH and FRAP assays. Figure 6 shows the

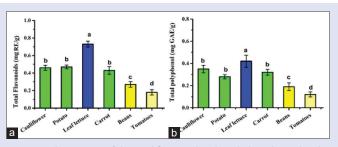


Figure 4: The content of the total flavonoid and total phenolic acid in the six purple plant

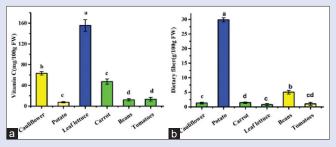
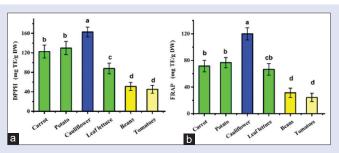


Figure 5: The content of the dietary fiber and Vitamin C in the six purple plants



**Figure 6:** The trolox equivalent antioxidant capacity value of the anthocyanin purified extracts from the six purple plants measured by 2,2-diphenyl-1-picrylhydrazyl and ferric reducing antioxidant power methods

antioxidant values of the APEs in the SPPs. The TEAC values of the APEs from purple cauliflower was found to be the highest,  $162.87 \pm 10.21$  mg TE/g DW and  $119.95 \pm 9.89$  mg TE/g DW based on DPPH and FRAP assay, respectively. This was followed by purple potatoes and the purple tomato, which was consistent with the results of TA content.

According to our results, there was a significant positive correlation between the content of TA and the antioxidant activity [Table 1 and Figure 6]. The ability to scavenge free radicals increases as the content of TA increases, the results of which are in agreement with previously reported data.<sup>[28-31]</sup> In this way, people can use color as one of the conditions for screening plants with high antioxidant capacity. There was a significant correlation between DPPH and FRAP. At present, it is well known that the nutritional value of black plants such as purple cauliflower, purple potato, and purple lettuce are higher than that of colorless plants. These plants show good anti-aging and anti-inflammatory activity, and protect the cardiovascular and cerebrovascular system from damage, all of which owe to the high content of anthocyanins. The darker the color is, the higher the TA content is, which in turn shows stronger antioxidant activity.<sup>[32,33]</sup>

provides a basis for further pharmacological research and clinical applications of purple plants.

### CONCLUSION

The experimental results showed that the SPPs were rich in anthocyanins, total flavonoids, and total polyphenols and they have strong antioxidant activity. The content of the TA in the APEs from the purple cauliflower was the highest, and it showed the strongest antioxidant activity as well. The content of flavonoids, polyphenols, and Vitamin C in purple lettuce was the highest, and the content of the dietary fiber in purple potato was the most abundant. SPPs are not only rich in anthocyanins, but also rich in functional ingredients. The results of the study provide a theoretical basis for the further exploration of the pharmaceutical value of purple plants and developing their applications.

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#### Conflicts of interest

There are no conflicts of interest.

#### REFERENCES

- Wyckoff EP, Evans BC, Manasse SM, Butryn ML, Forman EM. Executive functioning and dietary intake: Neurocognitive correlates of fruit, vegetable, and saturated fat intake in adults with obesity. Appetite 2017;111:79-85.
- Li H, Deng Z, Zhu H, Hu C, Liu R, Young JC, et al. Highly pigmented plants: Anthocyanin compositions and their role in antioxidant activities. Food Res Int 2012;46:250-9.
- Fallah AA, Sarmast E, Fatehi P, Jafari T. Impact of dietary anthocyanins on systemic and vascular inflammation: Systematic review and meta-analysis on randomised clinical trials. Food Chem Toxicol 2020;135:110922.
- Braga MB, Veggi PC, Codolo MC, Giaconia MA, Rodrigues CL, Braga AR. Evaluation of freeze-dried milk-blackberry pulp mixture: Influence of adjuvants over the physical properties of the powder, anthocyanin content and antioxidant activity. Food Res Int 2019;125:108557.
- Gowd V, Jia Z, Chen W. Anthocyanins as promising molecules and dietary bioactive components against diabetes – A review of recent advances. Ternds Food Sci Tech 2017;68:1-3.
- Goñi I, Díaz-Rubio ME, Pérez-Jiménez J, Saura-Calixto F. Towards an updated methodology for measurement of dietary fiber, including associated polyphenols, in food and beverages. Food Res Int 2009;42:840-6.
- Zhang Y, Li H, Shu W, Zhang C, Ye Z. RNA interference of a mitochondrial APX gene improves vitamin C accumulation in tomato fruit. Sci Hortic Amsterdam 2011;129:220-6.
- Choi Y, Lee SM, Chun J, Lee HB, Lee J. Influence of heat treatment on the antioxidant activities and polyphenolic compounds of Shiitake (*Lentinus edodes*) mushroom. Food Chem 2006;99:381-7.
- Popović BM, Stajner D, Slavko K, Sandra B. Antioxidant capacity of cornelian cherry (*Cornus mas* L.)-comparison between permanganate reducing antioxidant capacity and other antioxidant methods. Food Chem 2012;134:734-41.
- Li H, Deng Z, Zhu H, Hu C, Liu R, Young JC. Highly pigmented plants: Anthocyanin compositions and their role in antioxidant activities. Food Res Int 2012;46:250-9.
- Ieri F, Innocenti M, Andrenelli L, Vecchio V, Mulinacci N. Rapid HPLC/DAD/MS method to determine phenolic acids, glycoalkaloids and anthocyanins in pigmented potatoes (*Solanum tuberosum* L.) and correlations with variety and geographical origin. Food Chem 2011;125:750-9.

- Lachman J, Hamouz K, Musilová J, Hejtmánková K, Kotiková Z, Pazderů K, et al. Effect of peeling and three cooking methods on the content of selected phytochemicals in potato tubers with various colour of flesh. Food Chem 2013;138:1189-97.
- Barbieri G, Bergamaschi M, Franceschini M, Barbieri G. Feasibility of addition of polyphenol-rich vegetable extracts in whole cooked products: Benefits and drawbacks. Meat Sci 2018;139:1-6.
- Zhang L, Tu Z, Xie X, Lu Y, Wang Z, Wang H, *et al*. Antihyperglycemic, antioxidant activities of two *Acer palmatum* cultivars and identification of phenolics profile by UPLC-QTOF-MS/MS: New natural sources of functional constituents. Ind Crop Prod 2016;89:522-32.
- 15. Silva CL, Haesen N, Camara JS. A new and improved strategy combining a dispersive-solid phase extraction-based multiclass method with ultra high pressure liquid chromatography for analysis of low molecular weight polyphenols in plants. J Chromatogra 2012;1260:154-63.
- Kashyap D, Sharma A, Tuli HS, Sak K, Garg VK, Buttar HS. Apigenin: A natural bioactive flavone-type molecule with promising therapeutic function. J Funct Foods 2018;48:457-71.
- Al-Matani SK, Al-Wahaibi RN, Hossain MA. Total flavonoids content and antimicrobial activity of crude extract from leaves of *Ficus sycomorus* native to Sultanate of Oman. Karbala Int J Mod Sci 2015;1:166-71.
- Cheng CH, Liang HY, Luo SW, Wang AL, Ye CX. The protective effects of vitamin C on apoptosis, DNA damage and proteome of pufferfish (*Takifugu obscurus*) under low temperature stress. J Therm Biol 2018;71:128-35.
- Kory RC, Callahan R, Boren HG, Syner JC. The Veterans Administration-Army cooperative study of pulmonary function. I. Clinical spirometry in normal men. Am J Med 1961;30:243-58.
- Marik PE. Vitamin C for the treatment of sepsis: The scientific rationale. Pharmacol Ther 2018;189:63-70.
- Bender AB, Speroni CS, Moro KI, Morisso FD, Dos Santos DR, Da Silva LP. Effects of micronization on dietary fiber composition, physicochemical properties, phenolic compounds and antioxidant capacity of grape pomace and its dietary fiber concentrate. LWT Food Sci Techonol 2020;117:108652.
- Kosmala M, Zduńczyk Z, Karlińska E, Juśkiewicz J. The effects of strawberry, black currant, and chokeberry extracts in a grain dietary fiber matrix on intestinal fermentation in rats. Food Res Int 2014;64:752-61.
- Liu S, Jia M, Chen J, Wan H, Dong R, Nie S, *et al.* Removal of bound polyphenols and its effect on antioxidant and prebiotics properties of carrot dietary fiber. Food Hydrocolloid 2019;93:284-92.
- Joshi R, Kumar MS, Satyamoorthy K, Unnikrisnan MK, Mukherjee T. Free radical reactions and antioxidant activities of sesamol: Pulse radiolytic and biochemical studies. J Agric Food Chem 2005;53:2696-703.
- Mooren FC, Golf SW, Völker K. Effect of magnesium on granulocyte function and on the exercise induced inflammatory response. Magnes Res 2003;16:49-58.
- Wang S, Xia P, Wang S, Liang J, Sun Y, Yue P, et al. Packaging films formulated with gelatin and anthocyanins nanocomplexes: Physical properties, antioxidant activity and its application for olive oil protection. Food Hydrocolrloid 2019;96:617-24.
- Kalisz S, Oszmiański J, Hładyszowski J, Mitek M. Stabilization of anthocyanin and skullcap flavone complexes – Investigations with computer simulation and experimental methods. Food Chem 2013;138:491-500.
- Zhang YH, Liu N, Zhu WM, Guo SR. Extraction conditions and stability analysis of purple tomato anthocyanin. J Food Biotechnol 2018;37:88-92.
- Zhang J, Cheng WD, Shi XW, Xu YN, Zhang HM. Study on extraction technology and antibacterial activity of purple carrot *in vitro*. Chin Seasoning 2016;41:140-4.
- Xu H. Isolation, purification and component analysis of anthocyanin from purple cauliflower. Chin Food Addit 2017;19:112-6.
- Wang LJ, Su S, Wu J, Du H, Li SS, Huo JW, et al. Variation of anthocyanins and flavonols in Vaccinium uliginosum berry in Lesser Khingan Mountains and its antioxidant activity. Food Chem 2014;160:357-64.
- Giovanelli G, Buratti S. Comparison of polyphenolic composition and antioxidant activity of wild Italian blueberries and some cultivated varieties. Food Chem 2009;112:903-8.
- Luo Y, Cai RR, Fang JL, Yuan K. Total anthocyanins and cyanidin-3-O-glucoside contents and antioxidant activities of purified extracts from eight different pigmented plants. Pharmacogn Mag 2019;14:124-9.